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Effects of Ozone and Soil Water Deficit on Roots and Shoots of Field-Grown Soybeans

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Effects of Ozone and Soil Water Deficit on Roots and Shoots of Field-grown Soybeans

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ABSTRACT

*Water-stressed and well-watered soybean (*Glycine max *cvs. Williams and Corsoy) plants were exposed to increasing seasonal doses of ozone (O_3) using open-top field chambers and ambient air plots. Chamber O_3 treatments included charcoal filtered (CF) air, non-filtered (NF) air, NF + 0·03, NF + 0·06 and NF + 0·09 µl litre^{-1} O_3. Soil water potentials measured at 25 and 45 cm averaged −0·40 MPa and −0·05 MPa, respectively, for the plots in the water-stressed and well-watered series. Total root length/core, root length densities, and biomasses (dry weights) were determined. With Williams, a very popular cultivar in recent years, total root length for all O_3 treatments averaged 58% more under water-stress conditions than in well-watered plots, but the range was from 136% to 11% more for NF air and NF + 0·09 µl litre^{-1} O_3, respectively. Increasing the O_3 exposure dose did not affect root lengths or weights in the well-watered series. With Corsoy, water stress did not significantly increase root development. In both soil moisture regimes, with both cultivars, there was a linear decrease in seed yield and top dry weight as the O_3 exposure dose increased.*

INTRODUCTION

Although considerable information was available about the influence of soil water content and various chemical and physical factors on root growth and distribution (Pearson, 1971), in 1983, when this research was initiated, there was no information about the effects of ozone (O_3) exposure of the shoots on

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root development of field-grown plants. There was no information, also, about the combined effects of water stress and O_3 stress on root development under field conditions. The effects of water stress, as well as O_3, on roots are indirect since water use efficiency is determined largely by leaf characteristics.

Experiments conducted in controlled environments had shown that the root growth of soybean (*Glycine max*, Merr.) and radish (*Raphanus sativus* L.) was reduced when exposed to elevated O_3 in the seedling stage (Reinert *et al.*, 1971; Tingey *et al.*, 1971). Soil water deficits normally increase root length and decrease shoot growth (Meyer & Anderson, 1941). With soybean, increased root growth under drought conditions was reported in studies conducted in Australia (Burch *et al.*, 1978), Alabama (Huck *et al.*, 1983), Iowa (Mason *et al.*, 1982; Reicosky *et al.*, 1982), and Kansas (Mayaki *et al.*, 1976). However, on a fine sandy soil in Florida, root growth of soybeans was not significantly different in irrigated and non-irrigated plots (Robertson *et al.*, 1980).

Recently, the combined effects of O_3 and water stress on plants have received attention. Amundson *et al.* (1986) reported, when plants were grown in pots in environmental control growth chambers, the reduction in the growth of soybeans due to O_3 exposure was less for water-stressed than for well-watered plants. In the above experiment, Reich *et al.* (1985) measured leaf diffusive conductance and reported no interactions among the factors O_3, leaf age, or water stress. Temple *et al.* (1985) conducted a field experiment with open-top chambers to determine the responses of normally irrigated and water-stressed cotton (*Gossypium hirisutum* L.) to increasing O_3 exposure doses. In 1981, a typical hot, dry growing season in California, water-stressed plants showed almost no response to O_3 although yields of normally irrigated cotton were reduced 20% by ambient O_3 in the non-filtered chambers. However, in 1982, when weather was cooler and cloudier than normal, cotton yields in response to O_3 were similar for water-stressed and normally irrigated cotton. Irrigations were based on leaf water potential (ψ_l). Plants were furrow-irrigated when the ψ_l of water-stressed plants averaged $-2{\cdot}1$ MPa and in the normally irrigated plots when the ψ_l averaged $-1{\cdot}8$ MPa. Tingey *et al.* (1982) studied the influences of water stress on O_3 sensitivity of bean (*Phaseolus vulgaris* L.). Plants were grown in special containers which facilitated the development of a range of water-stress regimes. Water stress decreased plant sensitivity to O_3. Plants were no longer sensitive to O_3 when grown at $-0{\cdot}08$ MPa soil water potential (ψ_s) for 3 days.

The relationships between ψ_s values and measurable plant water stress are different for plants in pots and those grown under field conditions because of the normal gradient in ψ_s with soil depth in the field and the lack of such a

gradient in small containers. When soybeans were grown in 1·25 litre pots, Krizek *et al.* (1985) found ψ_s values were −0·4 to −0·6 MPa lower than those of controls if grown at −0·055 to −0·065 MPa ψ_s for only a week. When plants were grown at these ψ_s for 3 weeks, they had only about half the dry matter of the controls. The controls were maintained with 0 ψ_s by irrigation with a nutrient solution 2 to 3 times daily.

Heggestad *et al.* (1985) found greater than additive reductions in soybean plant growth and seed yields in field experiments in 1982 and 1983 investigating the combined effects of stress from exposure to ambient O_3 and soil water deficits. At depths of 25 and 45 cm the ψ_s averaged −0·05 MPa and about −0·40 MPa in well-watered and water-stressed plots, respectively. The primary objective of the current study, initiated in 1983, was to determine effects of increased O_3 exposure dose and water stress, singly and in combination, on soybean root development under field conditions. Seed yields and total plant dry weights at harvest of seeds are provided to permit comparisons of root and shoot growth.

MATERIALS AND METHODS

These investigations were part of a field experiment conducted in 1983 at the Agriculture Research Center, Beltsville, Maryland (Heggestad *et al.*, 1984, 1985). The soil type was a cordorous silt loam located on the flood plain of Paint Branch Creek. Gravel underlies the top soil at depths of about 1·2 m. The soil permits deep penetration by roots. According to the Prince Georges County, Maryland, soil survey (Kirby, 1967) the water table is seldom below 1·2 m, even in long dry periods. The bulk density, pH, and soil texture profiles for the 1·2 m soil horizon are provided (Table 1). Soil particle size was determined by the hydrometric method (Day, 1965). A 5-15-30 (NPK) fertiliser (500 kg ha^{-1}) and 1·75 litre ha^{-1} of trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine were applied prior to seeding.

Thirty cylindrical (3 m diameter) open-top field chambers (Heagle *et al.*, 1973) were employed to permit treatments with increasing O_3 exposure doses. In addition, six ambient air (no chamber) plots were included. Each plot contained one row of the soybean (*Glycine max*) cultivars Williams 79 and Corsoy 79, which are in maturity groups III and II, respectively. The cultivars were located at random on the east or west side of the plots. The width between rows was 0·9 m. Plots were seeded on 9 June after seed treatment with four USDA isolates of *Bradyrhizobium*. About five days after emergence, plants were thinned to an average spacing of 5 cm. The centre 2 m section of the 3 m rows was harvested, leaving 0·5 m of row at each end as plot border.

TABLE 1
Soil Bulk Density, pH, and Percentages of Sand, Clay, and Silt at 0·2 m Increments to a Depth of 1·2 m[a,b]

Depth (m)	Bulk density ($g\ cm^{-3}$)	pH	Soil texture		
			Sand (%)	Clay (%)	Silt (%)
0–0·2	1·39 ± 0·03	5·8 ± 0·14	39·9 ± 1·2	21·1 ± 0·7	39·0 ± 0·7
0·2–0·4	1·42 ± 0·04	5·8 ± 0·14	36·3 ± 1·4	21·1 ± 0·9	42·7 ± 1·0
0·4–0·6	1·42 ± 0·05	5·8 ± 0·15	36·8 ± 5·8	25·0 ± 2·7	38·2 ± 3·7
0·6–0·8	1·45 ± 0·05	5·3 ± 0·23	33·6 ± 4·3	29·8 ± 3·4	36·7 ± 1·9
0·8–1·0	1·52 ± 0·05	4·9 ± 0·20	39·8 ± 6·9	26·2 ± 3·0	34·0 ± 5·0
1·0–1·2	1·57 ± 0·08	4·6 ± 0·08	38·2 ± 4·2	31·1 ± 1·6	30·7 ± 3·0

[a] Means followed by standard error of means.
[b] Hydrometer method was used to analyse for sand, silt, and clay (Day, 1965).

O_3 treatments

Five O_3 exposure treatments were evaluated (CF air, NF air, and NF air to which 0·03, 0·06, or 0·09 μl litre^{-1} O_3 was added, designated as NF + 0·03, NF + 0·06 and NF + 0·09, respectively). Plots not enclosed by chambers were designated as ambient air (AA). The blowers for all chambers were turned on 12 July when plants were about 0·2 m in height and in the V3 vegetative stage of growth (Fehr *et al.*, 1971). In order to allow dew formation at night, blowers were off each day between 2100 and 0500 h (EST). Ozone treatments in the NF chambers were started on 23 July. These treatments were made from 0900 to 1600 h on 60 days of the subsequent 63-day period which were required before all plants in each plot had reached physiological maturity, R7, or were more mature.

The O_3 concentration in all (36) plots was monitored sequentially with TECO model 49* (Thermo Electron Corp., Hopkinton, Maryland) and Dasibi model 1003 PC (Dasibi Environmental Corp., Glendale, California) analysers. The analysers were calibrated weekly using a Dasibi 1003 PC ultraviolet calibrator. Sample air was drawn through Teflon tubing (0·63 cm OD) with the air intakes in the centre of each plot, about 5 cm above the canopy. The air sampling intake tubes were raised about every 10 days until the plants reached full height.

* Mention of trade or company names does not constitute a guarantee or a warranty of the product by the US Department of Agriculture and does not imply their approval to the exclusion of other products that may be suitable.

Soil water

Soil water potentials were monitored at 25 and 45 cm depths in all plots. Tensiometers (Irrometer Co. Inc., Riverside, California) were used to monitor ψ_s in plots without a water deficit. Tensiometers are effective in the range −0·02 to −0·08 MPa. Tensiometers were calibrated before use and on alternate weeks during the season. The tensiometers were serviced daily to maintain appropriate solution levels. Soil psychrometers (WESCOR PCT-55-15, Wescor Co., Logan, Utah) were used in the water stress series. These psychrometers are effective between −0·15 and −2·0 MPa. They were calibrated prior to installation using the dewpoint mode of operation. In the well-watered plots, ψ_s values were recorded each morning and used to determine the amount of irrigation water to apply, if any, to each plot. Water was applied at the rate of 0·5 cm per hour trickle irrigation (AGRIFIM Drip Irrigation, Shemin Nurseries, Inc., Burtonsville, Maryland). The water addition lines were positioned 15 cm from the plants on both sides of each row. An effort was made in 1983 to maintain about the same ψ_s as was used in the two soil moisture regimes in 1982 (Heggestad *et al.*, 1985). In plots without water, stress irrigation was initiated when the ψ_s reached −0·05 MPa at the 25 cm depth. In plots with water stress, irrigation was initiated usually when mean ψ_s values at the 25 and 45 cm depths reached −0·4 MPa. However, this varied slightly depending upon the rate of water use in the plots.

A maximum of 1·5 cm H_2O per day per plot was applied in the moist series and 0·5 cm H_2O per day per plot in the drought series. The addition of more than 1·5 cm of water per day resulted in water run-off. In the moist plot series there were plots watered on 45 days of the 67-day period from 18 July to 23 September. Measurable rainfall occurred on 13 additional days. In the water stress series, no water was applied until 3 August. During August and September these plots were irrigated on only nine days. However, on each date only plots with ψ_s less than about −0·4 MPa at the 25 to 45 cm depth were irrigated.

About 2/3 of the rain was excluded from all plots; i.e. well-watered plots as well as those with a water deficit. Clear plastic sheeting was placed over the central 2/3 portion of the 0·9 m wide area between the plant rows. An 0·3 m width in each row was left uncovered. To minimise the possibility of temperature increases beneath the plastic, in the 2 m harvested portions of each plot, the plastic was raised; i.e. it was attached to two light wood frames which were supported about 10 cm above the soil. In the plot borders the plastic was on the soil surface. It was extended for 1 m beyond the chamber via a shallow furrow in the soil beneath the chamber. On both sides of the two-row plots, the plastic cover was extended beneath the chamber and

about 1 m from the chamber. Prior to placing the chambers over the plots, the soil level in the centre (2 m by 2 m) of the plot was raised to assure run-off of rain falling on the plastic sheeting. Automated thermocouple measurement of temperatures on the soil surface and at 5 and 15 cm depths were made continuously during the season. In the harvested plot area where the plastic was elevated to provide ventilation, there was no effect of the 0·6 m wide plastic strip between the rows on soil temperatures within the row. By mid-August when plants reached the R5 stage of maturity, the plant canopy provided significant shading of the plastic.

Soil water content profiles were obtained for all plots on 16 September. A 2 cm i.d. soil corer was used to sample the soil to a 1·2 m depth. One core was taken near the middle of the row of Williams about 5 cm from the base of plants. Standard procedures were used to determine gravimetric water and the soil bulk density. Volumetric water content was computed from the data.

Leaf water potentials were obtained from plants receiving comparable O_3 treatments in the two soil moisture regimes. Usually, tests were made in only one replication of each treatment. Measurements were made on 11 days between 5 August and 16 September using a Scholander pressure chamber (PMS, Corvallis, Oregon). Terminal leaflets taken from the fourth trifoliate from the stem apices of three plants per plot were sampled each time.

Root measurements

Techniques used for root recovery from the soil and root length and dry weight measurements have been described (Bohm *et al.*, 1977; Anderson, 1986). Root length density (RLD) profiles for the cultivar Williams were determined from one soil core in each of 36 plots taken 3 cm from the base of plants and near the middle of the plot. Beginning on 6 September when plants in treatments with the earliest maturing plants (i.e. treatments with the highest O_3 exposure dose and water stress) reached physiological maturity, cores were obtained with a steel tube (1 m × 7·6 cm i.d.) manually driven with a drop hammer (Giddings Machine Co., Fort Collins, Colorado). A ladder jack was used to lift the soil filled metal core (Foale & Upchurch, 1982). The cores were divided into 0·2 m sections. The coring continued over a 2-week period as plants with the different treatments reached physiological maturity.

Roots were separated from the soil by agitation of the soil core sections in a plastic container (2·5 litres) with water plus detergent (0·5 tbs litre^{-1}). A 2·5-litre plastic container with a 1 × 1 mm screen in the bottom was inserted about halfway into the other container. The screen supported the soil core sections in the water. The tightly covered containers were shaken for about 30 min, or until the soil passed through the screen leaving the roots and

debris. The method permitted recovery of roots as fine as 0·125 mm diameter (Anderson, 1986). Roots and debris were separated by washing on a 0·25 mm mesh screen. Roots were frozen in petri dishes until measured. The line intercept method (Newman, 1966; Marsh, 1971) was used to determine root length. The RLD values are expressed as cm cm^{-3} and root dry weights as mg cm^{-3}. Also, root data are presented as m per core and g per core (soil core volume = 4·54 litres). Roots were dried for 18 h at 65° C to obtain dry weights.

After seed harvest and the removal of the open-top chambers (18–21 October) cores were taken with a 4·4 cm i.d. steel tube inserted by a Giddings tractor-mounted hydraulic corer into the holes remaining from the previous coring. These cores, taken between 1·0 and 1·4 m, provided two deeper 0·2 m soil core sections for qualitative evaluations of root growth. Rocks made it impossible to take cores deeper than 1 m in two plots. Some of the core sections taken between 1·2 and 1·4 m were very moist, indicating the presence of a water table.

Yield

Plants from 2 m row sections of each cultivar in each plot were cut at their base and removed on 5–14 October as pods turned brown and leaves abscised. During November, after additional drying at room temperature, the pods were removed and the seeds were hand-threshed. They were dried for three additional weeks at room temperature. At the time weights were made, the seeds contained 6·5% moisture. Seed weights were adjusted later to reflect 13% moisture. When plants with mature seed were removed from the field, four plants were selected (one plant from each 0·5 m section of the 2 m harvested row) to obtain an estimate of total plant weight; i.e. pods plus stems and any remaining dried leaves. Plant weights were obtained in November just prior to seed threshing.

Statistical analyses

The experimental design was a completely randomised split plot with three replications (blocks) and 12 treatments. Whole plots received different soil moisture and O_3 treatments. The two cultivars were assigned to individual rows (subunits) within the chambers. Therefore, O_3, water stress, and O_3 × water stress interactions were tested against chamber variation. Cultivar differences and interactions were tested against within-chamber variation. Correlation coefficients and regression equations were determined to show the relationship of irrigation water applied to maintain targeted ψ_s values in both soil moisture regimes and the 7-h seasonal O_3

concentrations in the five chamber treatments. Also, they were used to show the relationship between O_3 exposure dose and root lengths and weights in each of the moisture regimes. Mean differences among the 12 treatments were evaluated by the Least Significant Difference (LSD) method.

RESULTS

General

The seasonal, 23 July to 23 September, mean 7-h (0900–1600 h EST) O_3 concentrations for each treatment are given in Table 2. The daily ambient 7-h and 24-h O_3 concentrations from 11 July to 23 September 1983 are available (Heggestad *et al.*, 1985). Mean daily ψ_s values for the moist and dry soils are shown in Fig. 1. From 3 August to 20 September ψ_s values fluctuated between −0·22 and −0·54 MPa under the dry soil conditions, but in the moist soil regime the daily ψ_s values remained relatively constant (Fig. 1). For the dry soil regime, an analysis of variance of ψ_s for the six O_3 treatments on each ψ_s measurement date represented by 16 vertical bars in Fig. 1 revealed a significant effect of O_3 treatment and date but no

TABLE 2
Soil Water Potentials and Amount of Irrigation Water Applied for Each O_3 Treatment under Well-watered and Water-stressed Conditions

Chamber,[a] *O_3 added*	*O_3 7-h day^{-1} 63-day (μl litre^{-1})*	*Well-watered*[b]		*Water-stress*[b]	
		Water applied (cm)	*Soil water potential (MPa)*	*Water applied (cm)*	*Soil water*[c] *potential (MPa)*
CF	0·019	41·9	−0·06	4·3	−0·45
NF	0·052	43·3	−0·06	2·6	−0·32
AA	0·056	23·8	−0·05	0·7	−0·33
NF + 0·03	0·079	35·1	−0·05	1·2	−0·39
NF + 0·06	0·107	28·9	−0·05	1·2	−0·36
NF + 0·09	0·132	27·3	−0·05	0·8	−0·34

[a] Targeted O_3 additions to NF chambers from 0900 to 1600 EST; CF = charcoal filtered air, NF = non-filtered air, AA = no chamber.
[b] Values for season, means of three replications. Soil water potentials were means from 25 and 45 cm depths. First irrigation in the well-watered series was on 18 July, and in the water-stress series, 3 August.
[c] According to a Duncan's Multiple Range Test, the ψ_s value for CF air is significantly lower than those for all other treatments.

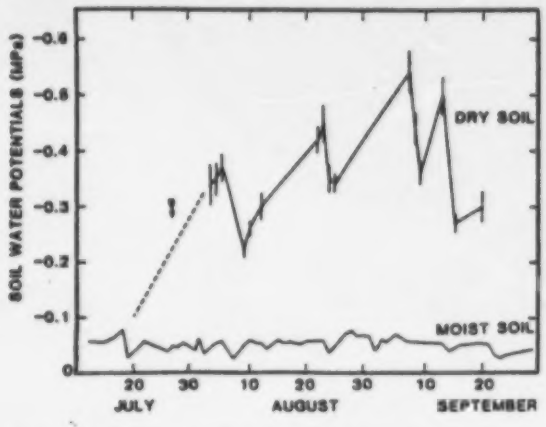

Fig. 1. Soil water potentials for two soil moisture regimes. A linear interpolation (arrow) is made between 17 July and 3 August for the dry soil conditions since neither tensiometers nor psychrometers measure accurately between −0·08 and −0·15 MPa. Plotted values are the average of the daily means for six treatments at 0·25 and 0·45 m depths in the three replications of each treatment. Vertical bars represent one standard error of the mean.

interaction between these variables. This indicates that the treatments had similar ψ_s on each date of measurement. The ANOVA also indicated that the mean ψ_s values at 25 and 45 cm (−0·34 and −0·39 MPa, respectively) were significantly different. The mean ψ_s values for each treatment and total amounts of irrigation water applied July through September at the two levels of soil moisture are shown in Table 2. In general, as the O_3 exposure dose increased in the chambers less irrigation water was required.

Corsoy began to flower on 11 July and Williams on 18 July. Williams in CF air with no water stress reached harvest maturity (R8) 12 October about one week later than Corsoy. Plants with water stress and exposed to NF + 0·09 μl litre^{-1} O_3 reached harvest maturity about two weeks before those exposed to CF air with no water stress.

Effects on root growth

Figures 2A and 2B show soil depth profiles of root length densities observed for plants (cv. Williams) give the various O_3 and water-stress treatments. Analysis of variance procedures revealed a highly significant ($P < 0·001$) interaction for root length between O_3, water stress, and soil depth. RLD values for CF and NF plots under water stress conditions were significantly greater than those for plants grown without water-stress. RLD was not affected by increases in the O_3 exposure dose in the moist soil series.

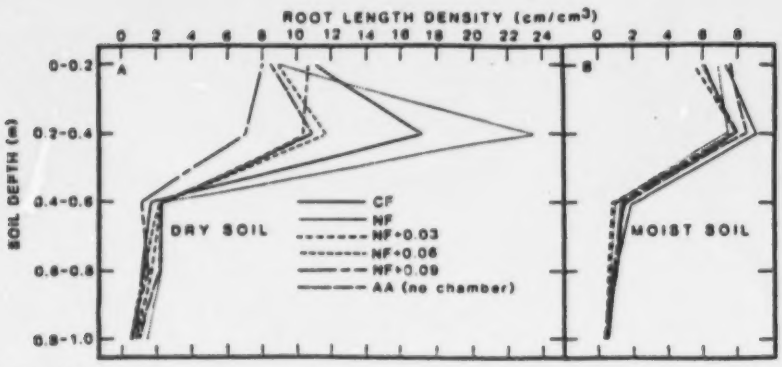

Fig. 2. (A) Effects of moderate level of water stress and increasing O_3 dose on root length density (RLD) of 'Williams', grown under field conditions; (B) effects on RLD in the absence of SMS. LSD at $P = 0{\cdot}05$, 3·2 when comparing RLD at various depths for each treatment, and 3·3 when comparing the RLD of 12 treatments at the same depth. CF = charcoal filtered air, NF = non-filtered air, and NF + 0·03, etc. indicates increases in O_3, $\mu l\,litre^{-1}$.

Maximum differences in RLD between the soil moisture regimes occurred at the 0·2–0·4 m depth. Examination of soil cores taken from 1·0 to 1·4 m indicated that in all treatments, with or without water stress, some roots occurred to a depth of 1·4 m (data not presented).

Because of the reduction in root lengths and weights in the soil cores as O_3 dose increased under only water deficit conditions, there was a significant

TABLE 3

Mean Square Comparisons for cv. Williams Showing the Effects of O_3 and Water Stress on Root Length and Root Weight in Soil Cores[a]

Sources of variation	*DF*	*Mean squares*	
		Root length/ core (m)	*Root weight/ core (g)*
Rep	2	6891*	0·51
O_3	5	6849*	1·01*
Water stress (WS)	1	74 301***	1·39***
O_3 × WS	5	6012*	0·67
Error	22	1983	0·36
R^2		0·78	0·68
CV		21·8	42·6

[a] Totals for roots in cores 7·6 cm D × 1 m taken 3 cm from the base of plants; three replications.
* $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

O_3 × water stress interaction for root length (Table 3). For the five chamber treatments there were significant linear correlations between O_3 dose and root length, and O_3 dose and root weight, under water-stress conditions, $r = -0.72$ and $r = -0.61$, respectively (Table 4). The regression equation for mean RLD in cm cm^{-3} in the cores is $Y = -29.0X + 7.9$ where $X = O_3$ concentration. The regression equation for mean root dry weight (mg cm^{-3}) in the cores is $Y = -3.46X + 0.70$. For Williams, based on the LSD values, the differences in root lengths in the cores between water stress and no water-stress conditions were significant for CF, NF and NF + 0·03 (Table 4). The root weights were significantly different between water stress and no water-stress conditions in only the CF and NF treatments. For Corsoy, with data from only the CF and NF treatments, there were no significant differences in either root lengths or weights (Table 4).

TABLE 4
Effects of O_3 on Root Length, and Root Weight with Plants Water-stressed (WS) and Well-watered (WW)

Cultivar[a] *treatment*	O_3 (7 h day^{-1}) (μl litre^{-1})	*Root length (m per core)*[b]		*Root weight (g per core)*[b]	
		WS	*WW*	*WS*	*WW*
Williams					
CF	0·019	293	185	2·58	1·16
NF	0·052	350	148	2·96	0·99
AA	0·056	228	174	1·42	0·90
NF + 0·03	0·079	236	141	1·53	0·89
NF + 0·06	0·107	225	156	1·49	0·93
NF + 0·09	0·132	167	151	1·08	0·90
LSD, $P = 0.05$		75		1·01	
r[c]		−0·72**	−0·23	−0·61*	−0·37
Corsoy					
CF	0·019	151	137	1·34	0·85
NF	0·052	102	108	0·93	0·72
LSD, $P = 0.05$[d]		79		1·21	

[a] CF = charcoal-filtered air, NF = non-filtered air, AA = no chamber, NF + = targeted O_3 additions to chambers from 0900–1600 EST, 63 days.

[b] Data are means for three replications; root values are for soil cores; core volume = 4·54 litres; to convert to root length in cm cm^{-3} divide core values by 45·4. LSD values may be used to compare any means within or between columns.

[c] Values for five chamber treatments; ** = $P < 0.01$, * = $P < 0.05$. Computed r with values for roots and O_3 in each replication.

[d] LSD values may be used to compare Williams and Corsoy in CF and NF air treatments, both within and between columns.

Soil moisture

On 16 September, when most plants were at the physiological maturity stage, the soil water contents were significantly different between plots with and without water deficit at all of the 0·2 m increments to a depth of 1·2 m (Fig. 3). Analysis of variance procedures indicated a highly significant ($P < 0{\cdot}001$) interaction between soil depth and soil water content whereas the interactions involving O_3 were not significant. This can be attributed to the application of irrigation water so as to maintain the same targeted ψ_s values for all O_3 treatments. The regression analyses of irrigation water applied to maintain targeted ψ_s values on 7-h seasonal O_3 concentrations for the treatments (Table 2) shows $r = -0{\cdot}68^{**}$ and $-0{\cdot}83^{**}$ ($^{**}P < 0{\cdot}01$) for chamber plots in the well-watered and water-stress series, respectively. The regression equations are $y = -150x + 46{\cdot}9$ and $y = -29{\cdot}3x + 4{\cdot}3$ for water-stressed and well-watered conditions, respectively; where $x = O_3$ concentration. The reduced soil water content from 0·6 to 1·2 m depth (Fig. 3) indicates that even with the low RLD (Fig. 2) at these depths roots were extracting water.

Plant water stress

Leaf water potentials ψ_l determined at midday on five days between 5 and 25 August ranged from $-0{\cdot}41 \pm 0{\cdot}02$ to $-0{\cdot}54 \pm 0{\cdot}01$ MPa in the water-stress

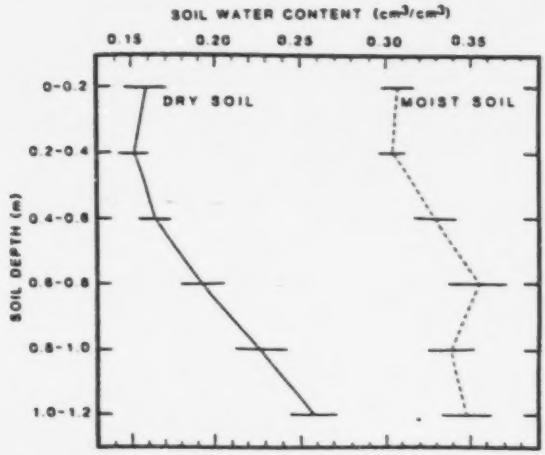

Fig. 3. Mean soil water content with depth, cv. Williams, for the six O_3 treatments under water stress conditions (dry soil), and no water stress (moist soil). Horizontal bars represent one standard error of mean. ANOVA indicated that the interaction of soil depth × water stress was significant, $P < 0{\cdot}001$; no interactions involving O_3 were significant.

regime. Mean values were only slightly greater for the same treatments in the well-watered regime. Measurements made between 30 August and 15 September, also on five days at about 1300 h, showed ψ_l decreases in the water-stress regime ranging from $-0{\cdot}87 \pm 0{\cdot}18$ to $-1{\cdot}88 \pm 0{\cdot}08$ MPa. Leaf water potentials were about the same in the well-watered regime. Early morning measurements (0715 h, EST) made on 16 September, however, gave lower ψ_l values for treatments under water-stress conditions than for the same treatments without water stress ($-0{\cdot}93 \pm 0{\cdot}16$ and $-0{\cdot}37 \pm 0{\cdot}04$ MPa, respectively) indicating only the plants grown with a water deficit remained under stress in morning hours.

Probably because of increases in root growth (Fig. 2A) and utilisation of water at greater depths (Fig. 3) our leaf water potential data taken at midday indicate little, if any, stress, occurred on healthy leaves of plants in both soil moisture series until late August. By 30 August the plants were beginning to show stress. At this time most of the plants were in the R5 maturity stage; i.e. beans were beginning to develop so they could be felt in the pods.

Cultivar root responses

Based on the available data, CF and NF air treatments, the root response of Williams and Corsoy to stress from soil water deficits were different. Analysis of variance procedures showed significant ($P < 0{\cdot}001$) cultivar × water stress × soil depth interactions for root length and weight (ANOVA Table not shown). Williams increased root growth significantly in response to water stress but Corsoy did not (Table 4). Williams had greater root length and root weight than Corsoy under water-stress conditions. For example, root lengths in NF air under water-stress conditions for Williams and Corsoy were 350 m and 102 m per 4·54 litres (a soil core) of soil (or, 7·72 and 2·25 cm cm^{-3}), respectively. Root dry weights in NF air under water-stress conditions for Williams and Corsoy were 2·96 g and 0·93 g per 4·54 litres of soil (or, 0·652 and 0·205 mg cm^{-3}), respectively. In the well-watered series, however, significant cultivar differences in root response were not found.

Yield

Bean yields (Table 5) and total plant dry weights at seed harvest (Table 6) are presented primarily to relate differences in root growth to those of the shoot. The seed yields are based on harvest of all plants (40 per plot) whereas shoot weights are based on only four plants per plot. The LSD values (Table 5) indicate significant differences in the Williams yields between each of the

TABLE 5

Effects of O_3 on Seed Yields of Williams and Corsoy with Plants Water-stressed (WS) and Well-watered (WW)[a]

Treatment[c]	O_3 7 h day^{-1} (μl litre^{-1})	Yield (g per plant)[b]			
		Williams		Corsoy	
		WS	WW	WS	WW
CF	0·019	29·4	34·7	26·4	26·8
NF	0·052	25·5	30·2	20·2	24·8
AA	0·056	25·2	22·6	18·2	19·6
NF + 0·03	0·079	23·6	24·6	19·2	16·3
NF + 0·06	0·107	21·4	19·0	15·8	12·4
NF + 0·09	0·132	16·4	15·7	13·9	12·0
r[d]		−0·98	−0·99	−0·98	−0·96
LSD, $P = 0·05$[e]		3·5 g			

[a] Means for three replications.
[b] Plant and row spacing permitted 218 700 plants per hectare.
[c] CF = charcoal-filtered air, NF = non-filtered air, AA = no chambers, NF + = O_3 additions in μl litre^{-1} to NF chambers from 0900–1600h EST, 63 days.
[d] The r values were computed using mean O_3 values and mean yields for the five chamber treatments.
[e] The LSD value may be used to compare mean values within and between the columns.

TABLE 6

Effects of O_3 on Shoot Weight at Harvest for Williams and Corsoy with Plants Water-stressed (WS) and Well-watered (WW)[a]

Treatment[b]	O_3 7 h day^{-1} (μl litre^{-1})	Shoot weight at seed harvest (g per plant)			
		Williams		Corsoy	
		WS	WW	WS	WW
CF	0·019	84·6 ± 5·7	103·1 ± 15·8	97·2 ± 4·3	121·3 ± 6·1
NF	0·052	51·8 ± 7·9	75·4 ± 11·2	59·3 ± 8·5	92·7 ± 16·8
AA	0·056	47·5 ± 1·2	49·8 ± 3·6	48·1 ± 4·7	58·0 ± 13·3
NF + 0·03	0·079	48·5 ± 3·9	46·3 ± 4·2	52·6 ± 9·1	56·2 ± 17·5
NF + 0·06	0·107	42·3 ± 1·8	41·5 ± 8·5	38·7 ± 5·9	32·1 ± 3·8
NF + 0·09	0·132	32·1 ± 3·3	36·5 ± 3·7	39·5 ± 5·3	31·3 ± 1·6

[a] Means followed by standard error of means; three replications, four plants in each replication.
[b] CF = charcoal-filtered air, NF = non-filtered air, AA = no chambers, NF + = O_3 additions in μl litre^{-1} to NF chambers from 0900–1600 h EST, 63 days.

chamber O_3 treatments when the plots were well-watered, but not under water-stress conditions. Williams yielded more than Corsoy for all treatments in the well-watered regime and for all treatments except NF + 0·09 in the water-stress regime. Correlation coefficient values for both cultivars in both dry and moist soil indicate a close linear relationship between O_3 dose and seed yield (Table 5).

Shoot weights (Table 6) provide an opportunity to examine shoot/root ratios using root weight data in Table 4. For Williams, these ratios decreased as the O_3 exposure dose increased in the well-watered regime; but, in the water-stress regime they were about the same except ratios for NF air were somewhat lower than for the other treatments.

DISCUSSION

Soybean plants may adjust to water stress resulting from reduced rainfall or irrigation water if there are significant amounts of stored water available at lower soil depths (Fig. 3). The adjustment to water stress by Williams at our field site involved a highly significant increase in root development as revealed by comparing RLD values in dry soil (Fig. 2A) with those in moist soil (Fig. 2B). The root environments in the experiments of Tingey *et al.* (1982), Temple *et al.* (1985) and Amundson *et al.* (1986) were very different from that in our field and from that of typical soils used for soybean production such as that described for Iowa by Mason *et al.* (1982). In the first two studies, the plants were grown in containers rather than as a field planting and in the latter study they were grown in a normally irrigated soil in California. In each experiment there was evidence that water stress protected plants from O_3 injury. Our bean yield data (Table 5) indicate that this did not occur in our experiment, especially with the NF treatment. At higher O_3 exposure doses, such as NF + 0·06, slight protection from O_3 injury is indicated. This difference in response to O_3 under our water-stress conditions may be due to differences in soil environments which affected root development. For example, root weights for the container-grown soybeans (Amundson *et al.*, 1986) showed that by the end of the season soybean plants in the well-watered regime had more than double the root weights of water-stressed plants. By contrast, under field conditions soybean root growth was usually increased under water-stress conditions, as shown by Burch *et al.* (1978), Huck *et al.* (1983), Mason *et al.* (1982), Mayaki *et al.* (1976), and Reicosky *et al.* (1982). Data on roots were not provided by Tingey *et al.* (1982) or Temple *et al.* (1985).

There was reduced root growth as the O_3 exposure dose increased under water-stress conditions, but the same O_3 treatments had no measurable

effect on root development when water stress was avoided (Fig. 2A and B). This result with Williams, a very widely-grown cultivar, is of considerable agronomic interest. The question may be raised whether the reduction in root growth, which seems related to increases in O_3 exposure dose, may have been due to differences in ψ_s. An analysis of variance involving ψ_s for each treatment on each date reflected by vertical bars in Fig. 1 revealed an effect of O_3 treatment and the date of ψ_s measurement but no interaction between these factors. Consequently, the ψ_s for the O_3 treatments on the different dates were not different. It seems that the difference in root development associated with O_3 dose under water-deficit conditions was an indirect effect of the O_3 exposure on the plant top. That is, O_3 decreased both seed yields and the weight of plant tops (Tables 5 and 6). Consequently, the need for root development to support plant growth was reduced. Effects of O_3 stress on root development may be accounted for in another way. Injury to the leaves by O_3 reduces photosynthate (Bennett & Hill, 1974). Any decrease in carbohydrates due to a diminution in the rate of photosynthesis reduces root growth as evidenced by increases in the shoot–root ratio (Meyer & Anderson, 1941). Reinert *et al.* (1971) and Tingey *et al.* (1971) were the first to show that root growth was depressed more by O_3 exposure than leaves. Okano *et al.* (1984) showed exposure of plants to an elevated O_3 dose reduced the amount of assimilates translocated to roots and stems by 53% and only 28% to leaves. These values obtained with *Phaseolus vulgaris* seedlings are useful but may be applicable only generally to soybeans in our field situation. We have no explanation as to why, under our conditions, higher than ambient O_3 exposure doses did not decrease RLD when moisture stress was avoided (Fig. 2B).

Our data on Williams (Figs. 2A and B) and other data (Mayaki *et al.*, 1976; Burch *et al.*, 1978; Mason *et al.*, 1982; Reicosky *et al.*, 1982; Huck *et al.*, 1983) showed that soybeans respond to water deficits by increasing root growth. Read & Bartlett (1972) have stated that, 'The most noticeable morphological response to controlled moisture deficit is the stimulated root growth.' They reported that small decreases in ψ_s can induce large increases in root–shoot ratios. In our water-stress series, regardless of O_3 exposure dose in the range of treatments given, the plants responded to the reduced ψ_s by increasing root growth, especially at the 0–0·4 m depth (Figs 2A and B).

For both cultivars there was a linear reduction in seed yield under both water stress and no water-stress conditions caused by the increase in O_3 exposure dose (Table 5 and Heggestad *et al.*, 1984). For Corsoy, a significant cubic response was indicated (Heggestad *et al.*, 1985). For Williams, the decreases in seed yield (Table 5) and root length (Table 4) under water-stress conditions from the lowest O_3 dose (CF air) to the highest (NF + 0·09) were about the same—namely, 44% and 43%, respectively; decreases in top

growth under water-stress conditions (Table 6) averaged 63% for the same treatments. In general, root growth reflects differences in top growth whether due to O_3 stress or other causes. Pearson (1971) stated that, 'Any condition that would affect top growth would indirectly influence root growth...'. However, if water stress is avoided less root growth seems required to sustain foliar growth (Fig. 2B). Although with Williams in the well-watered regime root lengths and root weights were about the same for all treatments (Table 4), the maximum O_3 dose caused a 55% decrease in seed yield and a 65% decrease in top growth. Because of the daily watering to maintain targeted ψ_s ($-0{\cdot}05$ MPa), apparently there was no stimulus to increase root growth above a minimum subsistence level.

Irrigation amounts required to maintain targeted ψ_s in the water-stress and well-watered regimes were reduced as the O_3 dose was increased in chambers with NF air (Table 2). The correlations between water applied to maintain targeted ψ_s and the O_3 dose in the chambers were $-0{\cdot}68$ and $-0{\cdot}83$ ($P < 0{\cdot}01$) under well-watered and water-stress conditions, respectively. In the well-watered regime plants in the no chamber (AA) plots required only 55% of the irrigation water applied to plants in the NF chamber plots to maintain targeted ψ_s levels. The need for water, also, was less since yields were 25% less than in the NF chambers (Table 5). Also, under water-stress conditions, the AA plots required less water than NF plots to maintain the same ψ_s (Table 2), even though bean yields and plant weights for AA and NF treatments were about the same (Tables 5 and 6). Perhaps there was greater loss of water in the chambers due to increased air movement and some rain was lost by contact with chamber walls. Under water-stress conditions, the difference in water applied to AA and NF treatments was only 1·9 cm (Table 2).

A few roots of plants in all treatments in both soil moisture regimes extended to a depth of 1·4 m. Roots in the lower soil profile have an important function under water-stress conditions, since roots near the capillary fringe of the soil may absorb most of the available water (Reicosky *et al.*, 1972; Stone *et al.*, 1976). Because plants used water to a depth of 1·2 m (Fig. 3), those in the water-stress regime were under less water stress than would be indicated by the ψ_s measured at 25 and 45 cm. Adjustment to the soil depletion of water in upper soil layers included an increase in RLD with a decrease in top growth and seed yields perhaps as a consequence. A similar experiment, involving both O_3 and water stress at this site conducted in 1982 with ψ_s about the same as in 1983, showed more than additive reductions in leaf size and weight due to the combination of O_3 and water stress (Heggestad *et al.*, 1985).

As expected, differences in the two soil moisture stress regimes affected the soil water profile characteristics. At each increment of soil depth the water

content was significantly less in the water-stress regime (Fig. 3). Greatest differences occurred near the soil surface (0–0·6 m depth).

Our maximum RLD values of 11·3 and 23·5 cm cm^{-3} for Williams under water-stress conditions at the 0–0·2 and 0·2–0·4 m depths, respectively (Fig. 2A) are higher than those reported for soybeans by others; e.g. Brown *et al* (1985) showed a maximum of 8·9 cm cm^{-3} in the 0–0·16 m depth. Other investigators showed lower RLD values for soybeans (Reicosky *et al.*, 1972, 1982; Burch *et al.*, 1978; Willatt & Taylor, 1978; Robertson *et al.*, 1980). Maximum Corsoy RLD values were 6·9 and 6·8 cm cm^{-3} rather than 11·3 and 23·5 cm cm^{-3} as shown above for Williams. Consequently, it appears that our high RLD values are due in part to cultivar differences under the conditions of this experiment. Chamber effects were a contributing factor since, under water-stress conditions, root length in AA was only 65% of that in NF (Table 4). Other factors affecting root development may be the irrigation protocol, method of rain exclusion, soil type and conditions, sampling distances from the row, and the technology used to separate roots from soil and to make the root measurements.

Cultivar differences in root development were found when plants were exposed to water stress but not in the absence of water stress (Table 4). The data indicate that only Williams responded to water stress by increasing significantly RLD and root weight density. Other studies have shown that soybean cultivars vary in root morphology and in their response to water deficits (Raper & Barber, 1970; Kaspar *et al.*, 1978; Willatt & Taylor, 1978).

This is the first field study examining both the effects of water stress and O_3 stress, singly and in combination, on soybean root distribution. Different results might be obtained if the roots were unable to penetrate the subsoil and utilise water stored at greater depths as occurred under our experimental conditions (Fig. 3). Without the availability to plant roots of water stored at greater depths, plants would have less time to acclimate to water deficits in upper soil layers causing leaves to wilt and stomata to close, thus giving some protection from O_3 injury. Under such conditions seed yields would be reduced primarily because of increased water stress, and O_3 exposure presumably would have minimal effects. More research with these stresses is needed, using different cultivars and soil types, to determine the effects of the combined stresses on top growth and roots of field-grown plants.

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